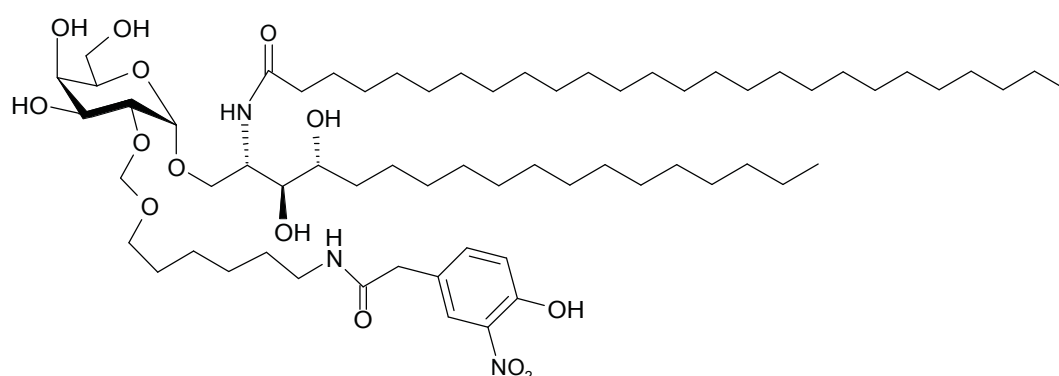


Invariant natural killer T cells direct B cell responses to cognate lipid antigen in an interleukin 21-dependent manner

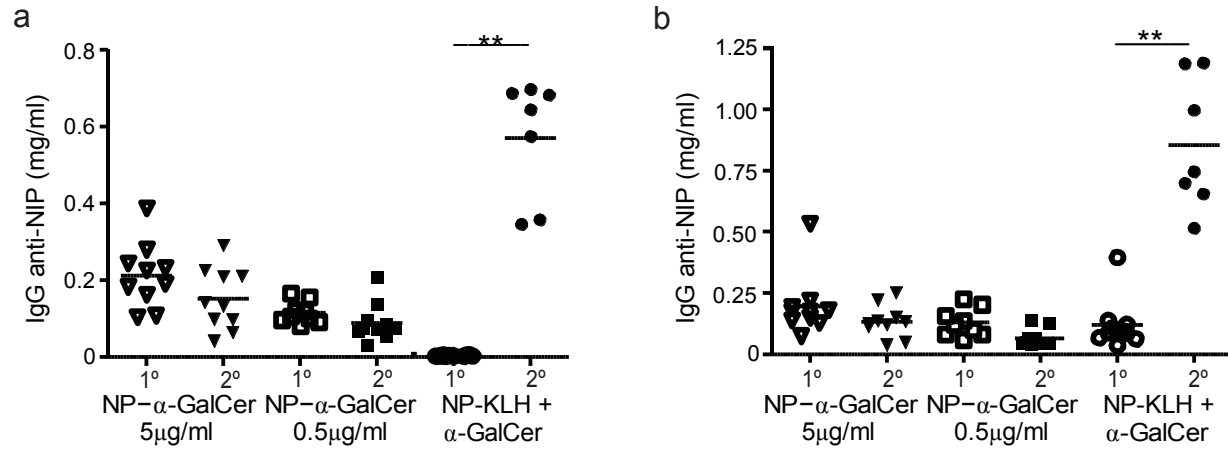
Irah L King, Anne Fortier, Michael Tighe, John Dibble, Gerald FM Watts, Natacha Veerapen, Ann M Haberman, Gurdyal S Besra, Markus Mohrs, Michael B Brenner, Elizabeth A Leadbetter

Supplementary Figure 1



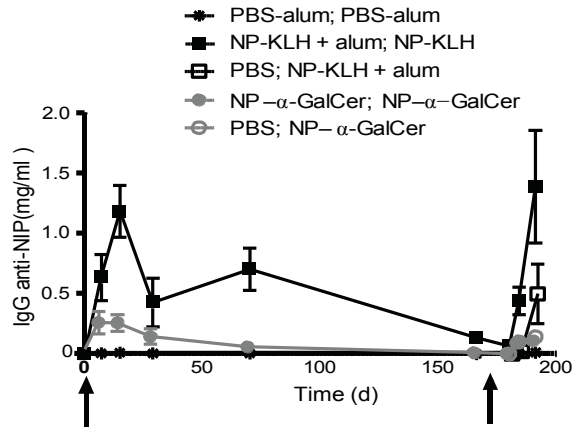
Supplementary Figure 1. Structure of NP- $\alpha$ -GalCer

Supplementary Figure 2



**Supplementary Fig 4: Neither high nor low dose lipid antigen induces a humoral memory response.** C57BL/6 WT mice were immunized IP on day 0 with 100  $\mu$ g NP-KLH plus 5  $\mu$ g  $\alpha$ -GalCer, 5  $\mu$ g NP- $\alpha$ -GalCer, or 0.5  $\mu$ g NP- $\alpha$ -GalCer and then received a secondary boost IV on day 51 with 100  $\mu$ g NP-KLH, 5  $\mu$ g NP- $\alpha$ -GalCer, or 0.5  $\mu$ g NP- $\alpha$ -GalCer (2°). Some mice received only a primary challenge at day 51 (1°). Each symbol represents an individual mouse following primary (1°) or secondary (2°) immunization at day 7 (a) and day 14 (b) post boost. Concentration of NP-specific IgG was determined by anti-NIP<sup>5</sup> ELISA. One experiment with 7-10 mice per group; each symbol represents an individual mouse; mean. \* $P$ =.006 \*\* $P$ =.0001 by Mann-Whitney test.

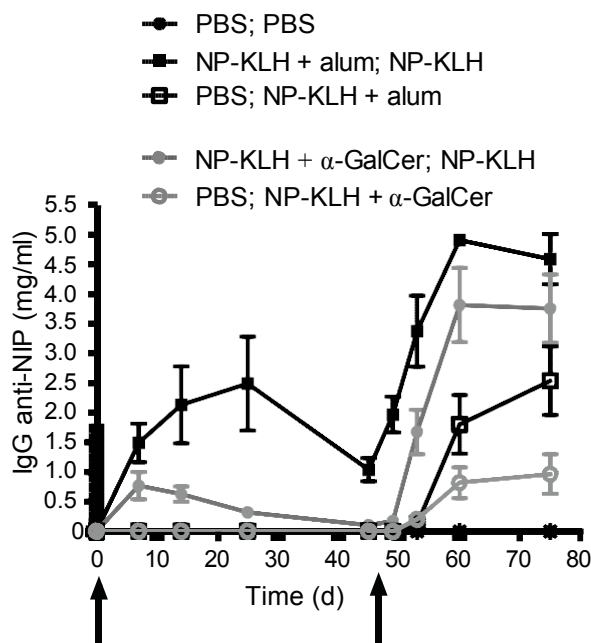
### Supplementary Figure 3



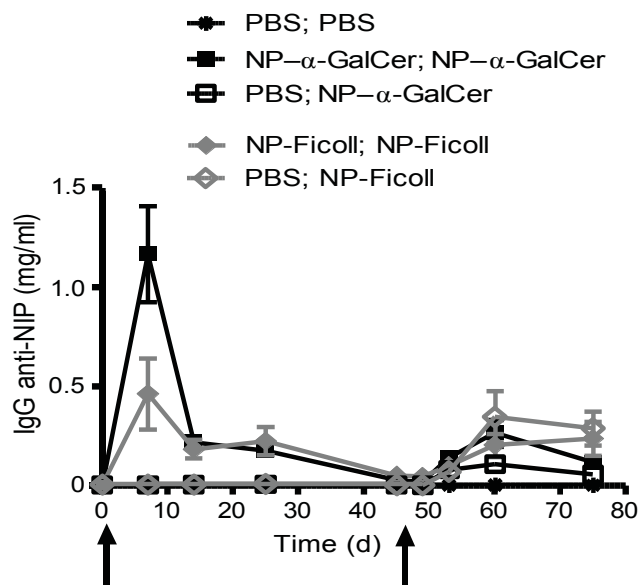
**Supplementary Fig 2: Only non-cognate *i*NKT cell help induces antibody memory response after day 177 rechallenge.** C57BL/6 WT mice were immunized IP on day 0 with PBS-alum, PBS, 2.2  $\mu$ g NP-KLH + alum, or 0.5  $\mu$ g NP- $\alpha$ -GalCer. Mice were bled periodically up to day 166. Mice then received a secondary boost IV on day 177 with 2.2  $\mu$ g NP-KLH in PBS (or IP with NP-KLH + alum as noted), 0.5  $\mu$ g NP- $\alpha$ -GalCer antigen, or PBS (see key) and were bled again on days 3, 7, and 14 post-boost. Key indicates primary challenge; secondary boost. Concentration of NP-specific IgG was determined by anti-NIP<sup>5</sup> ELISA. One independent experiment, with 10 mice per group. mean  $\pm$  s.d.

# Supplementary Figure 4

a



b



**Supplementary Fig 3: Only non-cognate iNKT cell help induces antibody memory response after day 46 rechallenge.** C57BL/6 WT mice were immunized IP on day 0 with 2.2  $\mu$ g NP-KLH in alum, 2.2  $\mu$ g NP-KLH plus 0.5  $\mu$ g  $\alpha$ -GalCer (a), 0.5  $\mu$ g NP- $\alpha$ -GalCer, or 30  $\mu$ g NP<sup>68</sup>-Ficoll (b) and were bled periodically up to day 45. Mice then received a secondary boost IP on day 46 with 2.2  $\mu$ g NP-KLH + alum, 0.5  $\mu$ g NP- $\alpha$ -GalCer, 30  $\mu$ g NP<sup>68</sup>-Ficoll, or PBS (as noted in key) and were bled again on days 3, 7, 14, and 29 post-boost. Key indicates primary challenge; secondary boost. Concentration of NP-specific IgG was determined by anti-NIP<sup>5</sup> ELISA. One independent experiment with 10 mice per group ; mean  $\pm$  s.d.